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Daily Rhythms of Feeding in the Genetically Obese and Lean Zucker Rats

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ALINGH PRINS, A., A. DE JONG-NAGELSMIT, J. KEIJSER AND J. H. STRUBBE. *Daily rhythms of feeding in the genetically obese and lean Zucker rats.* PHYSIOL BEHAV **38**(3) 423–426, 1986.—Feeding patterns were examined in obese (fa/fa) and lean (Fa/−) adult Zucker rats over the light-dark cycle during 14 days. Obese rats eat more than lean rats especially during the dark phase. Light and dark feeding expressed as percentage of 24 hr intake showed no significant differences between the lean and obese groups. The higher food intake in obese rats is mainly caused by larger meals since obese rats ate fewer meals than lean rats. Only for the obese group differences were observed between mean meal size in light and dark phase. There is some indication that the circadian controlled temporal distribution of meals is different in obese rats compared to lean rats since obese rats eat fewer but larger meals during the first half of the dark phase. During this phase meal size increases gradually in the obese rats, suggesting that the circadian influence on feeding motivation is increased.

Obesity   Meal pattern   Food intake   Feeding behavior   Circadian rhythm   Zucker rats

FOOD intake in wild and albino Wistar rats shows a light-dark rhythm, about 85% of the total intake being ingested in the dark phase [2, 7, 11]. In Wistar rats feeding activity during this phase shows two distinct peaks, one at the beginning and another towards the end of the dark phase. It has been reported that feeding behavior of obese Zucker rats is quite different from that of Wistar rats or even lean Zucker rats. They eat more than the lean Zucker rats [1, 3, 5, 6, 10]. They eat fewer but larger meals when given a liquid diet [1] but on solid food an equal number of meals [6,10]. They may even lack rhythmicity of feeding behavior [1].

These meal pattern studies may provide better understanding of the mechanisms underlying hyperphagia and obesity. However, most studies so far are designed for measures per light and dark phase or 24 hr.

Although mean values for 24 hr or for light and dark phase separately may give an overview, in actuality consideration of variation in the data used to calculate the means may also provide valuable information.

Since feeding motivation in the Wistar rats changes over the light-dark cycle it is feasible that some of the short term motivational signals in the hyperphagic obese rats dominate in certain parts of the light-dark cycle. For instance, it is known that in man an abnormal pattern of feeding behavior ('night eating syndrome') over the light-dark cycle may be an important causal factor in the occurrence of obesity [8]. The present study therefore investigates how feeding behavior expressed as total amount ingested, meal size and frequency varies over the light-dark cycle for obese and lean Zucker rats.

METHOD

Animals and Housing

Six adult male obese Zucker rats (fa/fa) and five lean littermates (Fa/−) were kept in two sound attenuated rooms. At the start of the experiment the rats were 10 months old. The rats were obtained from the Centre for Small Laboratory Animals of the Agricultural University of Wageningen (The Netherlands) where they were reared on normal lab chow.

The rats were housed individually in Plexiglas cages (40×40×40 cm) on a rigid wire mesh floor. Food pellets (Muracon, Trouw, Putten, The Netherlands) in metal hoppers and water were available ad lib. Food hoppers were filled regularly about twice a week and at varying times in the light phase. The animals were kept under an artificial light-dark (LD) regime (12:12 hr). For practical reasons the dark period was from 0–12 hr and the light period from 12–24 hr. The food consisted of 20% protein, 53.3% carbohydrate, 5% fat, 4.4% cellulose, 11.8% water and added minerals and vitamins. The room temperature was thermostatically controlled at 21±0.2°C.

Feeding Behavior

A rat could gnaw off pieces of food through vertical stainless steel bars situated in front of the hopper. Spillage was collected in an undertray attached to the food hopper. Each food hopper's weight was sampled by a programmed microprocessor every tenth of a second. Every second the mean weight was calculated and stored on a digital magnetic cas-

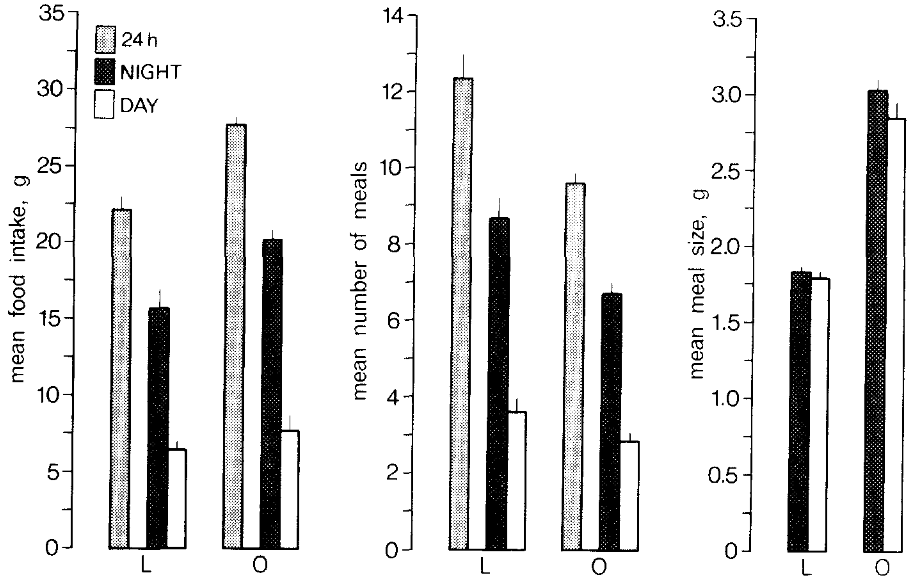

FIG. 1. Mean food intake (g), number of meals and meal size (g) per 24 hr and during the light and dark period (mean±SEM) in lean (L) and obese (O) Zucker rats.

sette tape if it was different from the previous weight. Cassette recordings were reduced by a program on a PDP-8 computer to retrieve the real size and timing of meals. An Esterline Angus event recorder was used to monitor feeding behavior directly, thereby obtaining a visual check on the computer data.

In defining a meal the intermeal interval must be taken into account. It has been reported that the ideal intermeal interval lies between 10 and 40 minutes for a rat eating ad lib [1]. We adopted an intermeal interval of 15 min, since this is a reasonable estimate and gave consistent results. There was no change in mean meal frequency when other intermeal intervals between 10 and 20 min were chosen. Thus feeding sequences which were separated from each other by intervals larger than 15 min were considered as separate meals.

Data Analysis

The following parameters were used for analysis: (1) The amount of food taken per hour, (2) The number of meals per hour and, (3) The meal size per hour. Further, the means of these parameters over the light and dark phase were calculated.

The data were evaluated first by a 3-way ANOVA, mixed model. Factors are named in the results section as GEN for genotype: lean or obese; LD for light or dark phase of the day; DAY for the fourteen days of observation and RAT for individuals. Of the latter two, RAT is subordinate to GEN and added little to the within group variance and DAY stands for repeated measures. Days in case of 12 hour intakes were made independent by taking one 12 hour period, omitting the next two 12 hour periods, taking the next period and omitting three of these periods, etc., to obtain equal numbers of independent light and dark periods. This leaves 132 periods of the original 308. All 14 days were used in case of the individual hourly intakes as these were regarded independent.

Because meals were more frequent during the dark than during the light a Mann-Whitney U-test was used to compare meal sizes.

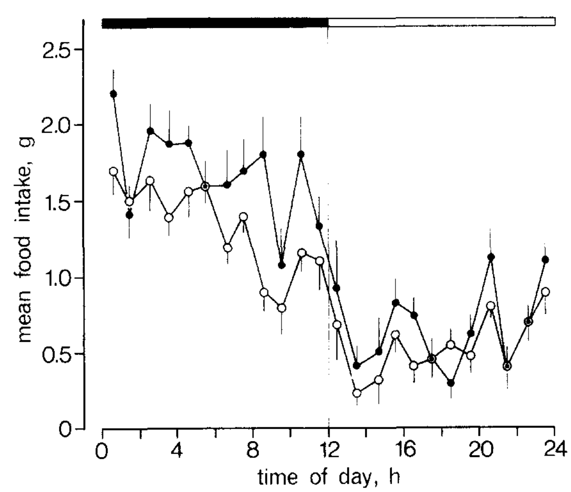

FIG. 2. Mean food intake (g) per hour over the whole light-dark cycle for lean and obese Zucker rats (mean±SEM). ○, lean; ●, obese.

RESULTS

During the experimental period, body weight of the obese group varied from 571±5 g in the beginning to 583±8 g (mean±SEM) after 14 days. For the lean group these measures were 368±6 g and 380±6 g respectively.

Food intake was higher in the obese group than in the lean group [GEN, $F(1,18)=10.1$, $p=0.005$] (Fig. 1). However, there was a small but significant interaction with the light-dark cycle [GEN by LD, $F(1,18)=4.76$, $0.05>p>0.025$]. Therefore the GEN effect was tested separately for both light and dark periods by a post hoc t-test: during the dark period the intake of lean rats (15.8 g/12 hours) was significantly less than that of obese rats (20.1 g/12 hours) [$t(64)=7.304$, $p\ll 0.001$]; during the light period intake of lean

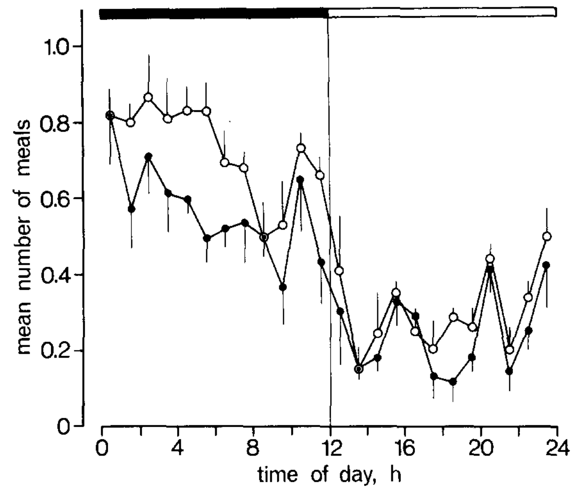

FIG. 3. Mean number of meals per hour over the whole light-dark cycle for lean and obese Zucker rats (mean±SEM). ○, lean; ●, obese.

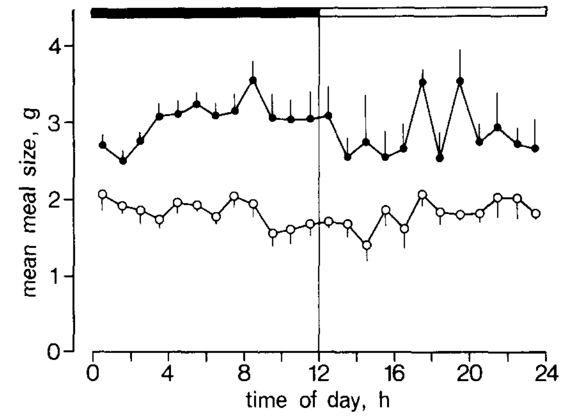

FIG. 4. Mean meal size (g) per hour over the whole light-dark cycle for lean and obese Zucker rats (mean±SEM). ○, lean; ●, obese.

rats (6.6 g), and obese rats (7.4 g) did not differ [$t(64)=1.659$].

Food intake was in both genotypes higher during the dark than during the light phase [LD, $F(1,18)=192.07$, $p<0.001$]. When food intakes during the dark and light phase were expressed in percentages of daily intake no significant differences between the lean and obese groups were observed (Fig. 1) (lean 71±4% for the dark period and 29±2% for the light period vs. 73±3% and 27±3% for the obese group).

During the dark phase the lean Zuckers ate more meals (8.7 meals/12 hours) than the obese rats (6.5 meals). During the light period the same difference was observed (3.6 and 2.8 meals respectively) [GEN, $F(1,18)=14.60$, $p<0.001$]. As before a small interaction with LD was found [GEN by LD, $F(1,18)=3.43$, $0.1>p>0.05$].

When separating the GEN and LD factors in post hoc t-tests, using independent (see the Method section) 12-hour measurements, this difference in meal numbers was confirmed. The lean animals ate more meals than the obese rats during the light period, $t(64)=3.73$, $p<0.001$, and during the dark period as well, $t(64)=6.07$, $p\ll 0.001$. In the dark period the number of meals for both groups was higher than during the light [3-way ANOVA, LD, $F(1,18)=104.99$, $p<0.001$]. When the number of meals during dark and light phase was expressed as percentage of total number in 24 hours no significant differences between the lean and obese group were observed (Fig. 1) (lean 70±6% and 30±2.5% vs. obese 71±2.8 and 29±2.5% respectively).

Meal sizes averaged over 12 hour periods differed slightly between light and dark phases, but in the obese group the average meal was much larger than in the lean group. The number of meals during the light period is much smaller than during the dark period. Therefore the meal sizes were compared by the Mann-Whitney U-test. During the dark periods obese animals ate larger meals of 3.06 g on the average than lean animals (1.86 g) ($n1=84$, $n2=70$, $U=9$, $p<0.001$). During the light phase these numbers were: obese 2.81 g and lean 1.84 g ($n1=84$, $n2=70$, $U=745$, $p\ll 0.001$) respectively. The differences between dark and light within each of the genotypes was significant only for the obese rats ($n1=n2=84$, $U=2772$, $p<0.015$).

Lean Zucker rats show a clear daily variation in food intake (Fig. 2). During the first half of the dark phase lean rats eat a relatively constant amount of food per hour. Thereafter a decrease occurs which is interrupted by a peak at the end of the dark phase between 10 and 12 hr. From then on feeding activity drops sharply in the early 2 hr of the light phase. During the light phase feeding behavior peaks between 15 and 16 hr, 20 and 21 hr and rises steeply to levels observed in the dark period between 23 and 24 hr. A similar distribution of peak feeding activity was seen in obese rats. In general the feeding activity of obese rats parallels feeding activity of the lean rats during large parts of the light-dark cycle.

At most hours of the light-dark cycle food intake of the obese rats is higher than that of the lean rats. In a two-factor ANOVA, (GEN by RAT) with repeated measures (DAY) on food intake per hour for each of the 24 hours a number of hours proved to be different on factor GEN ($df=1,126$). Obese rats ate significantly more during hours 1, 5, 7, 9 and 11 in the dark period and in hour 17 in the light period ($\alpha=0.05$, two sided). There is a strong similarity between the pattern of food intake and pattern of meal frequencies (Figs. 2 and 3). Meal frequency shows a comparable distribution in both groups, too (Fig. 3). At most times the meal-frequency-curve of the lean rats is above that of the obese rats. Significant differences in meal frequency were found in hours 2, 4, 5, 6, 7, 10 and 11 of the dark and once in hour 19 during the light, $\alpha(1,126)=0.05$.

As already described (Fig. 1) the main difference in feeding behavior of obese and lean rats is seen in meal size. Figure 4 shows that this is also true for many hours of the light-dark cycle. In a two-factor ANOVA (GEN by RAT) executed for all 24 hours on meal size per hour the GEN effect is significant in all hours except hour 2 in the dark and in hours 13, 16, 17, 18, 21 and 24 in the light [$\alpha(1,37)=0.05$; degrees of freedom were often much lower for the daytime hours since only those hours with a meal were taken into account]. Meal size of lean rats does not differ very much over the light-dark cycle. Just around light onset somewhat smaller meals are seen. Obese rats however show more fluctuations in their meal size. During the dark phase meal size increased gradually from 2.5±0.14 g in the first two hours to 3.6±0.24 g in hour 8–9. From then on a gradual decrease starts.

DISCUSSION

The present experiment shows that the obese Zucker rats eat more than lean rats mainly by increasing meal size during the light phase as well as during the dark phase. These experiments extend findings of others [1, 3, 5, 6, 10].

We have seen that food intake is higher in obese rats than in lean rats but the distribution over the light-dark cycle is the same. When feeding during the light and dark phases is expressed as percentages there is no significant difference between obese and lean rats. Frequency of meals was lower in obese rats, but again when expressed as percentages of total number of meals over the light and dark phase no significant difference was found. This indicates that the circadian influence causing day and night shifts in the expression of feeding behavior is similar in lean and obese rats. Others, however, reported a strong difference in rhythmicity between lean and obese rats [1]. Besides age differences, it is possible that in those experiments feeding on liquid food is responsible for the observed differences with the present study in which solid food is used.

There is increasing evidence that temporal distribution of meals is under circadian control [12,14]. It is suggested that the regulation of energy homeostasis may take place within the boundaries set by the circadian control [12]. Whereas the pattern of meals follows the circadian control of feeding over the light-dark cycle, meal size is probably involved in the adjustment of energy homeostasis. Several reports have shown that energy regulation is achieved primarily by changing meal size and not meal frequency. For instance in case of lower ambient temperature [4], diabetes [16], lactation [13] and refeeding after fasting [9], rats eat more food to compensate their energy deficit by increasing meal size. When very high energy intakes are required, i.e., lactating rats with a large litter, meal frequency is also increased [13]. Although meal size can be determined by factors governing energy homeostasis, some circadian influence on meal size cannot be excluded. We obtained evidence that in albino Wistar rats clock time can influence meal size. Mean meal size during daytime was smaller than in the dark phase [15]. In lean Zucker rats, however, meal size did not differ between light and dark phase. In contrast in obese Zucker rats meal size was slightly decreased in the light phase. In the genetically obese rats meal size increased gradually during the dark phase reaching a maximum between 8 and 9 hr. It is possible that some circadian influence causes this effect.

Although there is a strong similarity between the frequency of meals per hour of obese and lean rats, small differences occur in the first half of the dark phase. These results suggest that if there is a difference in circadian control of this aspect of feeding behavior, its influence is small. In comparison with Wistar rats which show a high level of feeding activity towards the end of the dark phase [7,12], the lean Zucker rats eat more during the first half than during the second half of the dark phase. Both lean and obese Zucker rats show at the end of the dark phase a peak in feeding activity. In our previous reports we presented evidence that this peak is under rigid control of a circadian oscillator [7, 12, 14, 15]. Besides this last peak in the dark phase, there are also striking similarities in the occurrence of peak feeding activities during the light phase. It is possible that these similar patterns of activities in obese and lean rats are caused by an ultradian oscillator which exerts its control in addition to the slower rhythm of the circadian oscillator controlling rhythmic expression of feeding behavior [17].

In summary, there is some indication that the circadian controlled temporal distribution of meals is different in obese Zucker rats compared to the leans. A gradual increase in meal size in the obese rats during the dark phase may suggest an increased circadian influence on feeding motivation in that phase.

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